Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Hwang et al., J Nutr Food Sci 2014, 4:5
DOI: 10.4172/2155-9600.1000295

Research Article Open Access

Nutritional Composition Changes in Skate (*Raja kenojei*) during Different Ripening Periods

Jae-Ho Hwang[1], Bok-Mi Jung[2], Sung-Ju Rha[1], Jin-Man Kim[3], Seon-Jae Kim[4] and Tai-Sun Shin[2*]

[1]College of Fisheries and Ocean Sciences, Chonnam National University, Yeosu 550-749, Korea
[2]Division of Food Nutrition Science, Chonnam National University, Gwangju 500-757, Korea
[3]Department of Biotechnology, Chonnam National University, Yeosu 550-749, Korea
[4]Department of Marine Bio Food, Chonnam National University, Yeosu 550-749, Korea

Abstract

In order to suggest fundamental data on the nutritional composition of the Korean traditional food, ripened skate, this study investigated the proximate composition, organic acid, free sugar, fatty acid, total amino acid, and macromolecule levels of skates at different ripening periods. Longer ripening periods resulted in decreased moisture levels and increased protein and ash levels. The total organic acid content increased until the 15th day of ripening, but decreased at 20 days. Phosphoric acid, oxalic acid, and lactic acid were the dominant organic acids. Eight free sugars were detected, ribose, glucose, and sucrose being the dominant forms, which significantly decreased with ripening. Palmitic and lignoceric acid were abundant in saturated fatty acids, oleic acid and palmitoleic acid were abundant in monounsaturated fatty acids, and linolenic acid, docosahexenoic acid, and arachidonic acid were abundant in polyunsaturated fatty acids. The major amino acids were glutamic acid, lysine, aspartic acid, and leucine and the total levels increased with ripening. The most abundant free amino acids were urea, sarcosine, taurine, and β-alanine, which decreased with ripening. Phosphoserine, phosphoethanolamine, α-aminobutyric acid, and cysteine levels increased.

Keywords: Ripening period; *Raja kenojei*; Organic acid; Free sugar; Fatty acid; Amino acid

Introduction

Skate (*Raja kenojei*) is a popular Korean cuisine. In the southwestern part of Korea, the preparation of a traditional dish for skate involves allowing the skate to ripen in a ceramic jar without any additives for over 1 week at room temperature, in contrast to products salted and fermented with rice-bran or rice such as Heshiko and Funazushi in Japan [1,2] and to salted product, Jeotgal in Korea [3]. This preparation is preferred because it results in an elastic texture of the dermal fin rays and a unique ammonia-like flavor derived from the large quantity of trimethylamine oxide and/or urea present in the preparation [4-6]. It is not toxic to humans, provided it is kept at the recommended temperature and humidity. According to experienced Korean cooks, the skate hardens during ripening; however, this seems to contradict general knowledge pertaining to raw fish storage, which indicates that softening occurs after only 1 day in chilled storage [7-9]. Both the shark and skate are elasmobranch fish and shark type II collagen has been reported to be an effective treatment against chronic arthritis [10]. As such, there is also a high possibility that ripened skate has the same therapeutic effects as type II collagen. Many studies have been performed on skates with respect to their classification [11], physicochemical changes [12,13], cholesterol and fatty acids [14], muscle, skin, and cartilage collagens [15-19]. Despite the interest in ripened skate, only a few studies have been published on this topic in some Korean local papers. To our knowledge, this is the first study to evaluate the differences in the nutritional composition of skate (*Raja kenojei*) during ripening periods.

Methods

Sample preparation

Skates (*R. kenojei*) were purchased from a market (Mokpo, Jeonnam, Korea) and divided into 5 groups (1.5 kg/group), specifically, 1 control and 4 different ripening groups (0, 5, 10, 15, and 20 days). The samples for each ripening group were sealed in 4 different ceramic jars and stored in a cold room at 8°C. The jars were selected randomly at 5 d intervals and the edible parts were separated from the body of the skate. The minced samples were divided into 3 groups (500 g/group) and stored at -80°C until use.

Proximate analysis

For proximate analysis [20] of the skate samples, the samples were dried in an oven, ground, and mixed for analysis in triplicate. The amount of crude protein was determined using the Kjeldahl method (Kjeltech auto sampler system 1,035 analyzer; Foss Co., Hillerød, Denmark) and the crude lipid content was determined using the ether-extraction method (Soxtec 2050 auto extraction unit; Foss Co.). The moisture level was determined using an auto moisture system (HR 73; Mettler Toledo Co., Grelfensee, Switzerland) and the ash content was determined using a muffle furnace (TMF-3100; EYELA Co., Tokyo, Japan) at 550°C for 4 h.

Organic acid and free sugar analyses

The standard organic acids and free sugars were prepared with a 1 mg/mL stock solution and quantified by diluting the stock solution. Skate samples (5 g) were homogenized in a tissue grinder (IKA, Germany) with 20 mL of 80% ethanol, and then reflux extracted in a water bath for 3 h by adding 80 mL of 80% ethanol in a 250 mL flask. The suspensions were centrifuged at 4000 ×*g* for 30 min, and filtered using

***Corresponding author:** Tai-Sun Shin, Division of Food Nutrition Science, Chonnam National University, Gwangju 500-757, Korea, Tel: +82-61-659-7415; Fax: +82-61-659-7415; Email: shints@chonnam.ac.kr

Received July 03, 2014; **Accepted** July 19, 2014; **Published** July 22, 2014

Citation: Hwang JH, Jung BM, Rha SJ, Kim JM, Kim SJ, et al. (2014) Nutritional Composition Changes in Skate (*Raja kenojei*) during Different Ripening Periods. J Nutr Food Sci 4: 295. doi: 10.4172/2155-9600.1000295

Whatman No. 1 filter papers (Whatman, NJ, USA). The filtered extracts were concentrated to approximately 1 mL by using a rotary evaporator (CCA 1110; Eyela Co. Ltd, Japan) and resuspended with a starting buffer to obtain a volume of 10 mL. The solution (3 mL) was then filtered with a 0.2-μm membrane and analyzed using high performance liquid chromatography (HPLC) (Prominence HPLC, Shimadzu Co. Ltd., Kyoto, Japan) for analyzes of organic acid and free sugar.

Measurement of free sugar content was carried out using HPLC. The HPLC equipment consisted of a LC-20AD HPLC pump, a Sil-20AC auto-sampler, a CTO-20AC oven, and a CBM-20A system controller (Shimadzu Co. Ltd., Kyoto, Japan). The columns used for analysis were an ion exchange Shim-pack ISA-07 analytical column (4.0 mm × 250 mm) and a Shim-pack ISA guard column (4.0 mm × 50 mm). The HPLC was operated at an oven temperature of 65°C with 0.6 mL/min flow of solution A (potassium borate, pH 8.0) and solution B (potassium borate, pH 9.0) for 90 min as the linear gradient elution method. 20 μl of hydrolysate filtered by membrane filter (0.45 μm) was injected. After separation of the saccharides by the column, the arginine/boric acid reagent solution is continuously added to the column eluent to convert the saccharides to fluorescent derivatives for detection [21]. The detector used was the RF-10Axl fluorescence detector set at 320 nm (Ex) and 430 nm (Em). The content of the total free sugars was calculated based on a standard curve while reducing the sugar standard material.

Measurement of the organic acid content was carried out using HPLC. The HPLC equipment consisted of a LC-20AD HPLC pump, a Sil-20AC auto-sampler, a CTO-20AC oven, and a CBM-20A system controller (Shimadzu Co. Ltd., Kyoto, Japan). The columns used for analysis were tandem ion exchange Shim-pack SPR-102H analytical columns (7.8 mm × 250 mm) and a Shim-pack SPR-H guard column (7.8 mm × 50 mm). The HPLC was operated at an oven temperature of 40°C with 0.8 mL/min flow of 4mM p-toluenesulfonic acid as the post-column method. 10 μl of hydrolysate filtered by a membrane filter (0.2 μm) was injected. 16 mM Bis-Tris aqueous solution containing 4 mM p-toluenesulfonic acid and 100 μM EDTA was used for the reactive reagent. The detector used was a conductive detector. The content of the total organic acids was calculated based on a standard curve with an organic acid standard material.

Analysis of fatty acids

Bligh and Dyer extraction was performed using the following method [22]: Briefly, lipids were extracted from 50 g samples by homogenization with 400 mL chloroform and 200 mL methanol. The samples were then filtered and evaporated to remove the solvent. Fatty acid methyl esters (FAMEs) were prepared using boron trifluoride (BF3) according to a method described by the association of official analytical chemists (AOAC) [20]. Quantitative analysis of FAME was carried out on a GC-2010 gas chromatography system (Shimadzu Co. Ltd.) equipped with a split/splitless capillary inlet system and a flame ionization detector (FID) using SP-2560 capillary columns (stationary phase thickness, 0.2 μm; 100 mm (length) × 0.25 mm (i.d.); Supelco, Inc., USA). The sample (0.5 μL) was injected in the split mode using an automatic injection system (AOC-20i; Shimadzu Co. Ltd.). The oven temperature was programmed to increase from 150 to 250°C at 3°C/min with initial and final holds of 5 min. The other operation parameters were as follows: injector temperature=270°C, detector temperature=250°C, helium carrier gas flow=18 cm/s, and split ratio=1:50. The peak areas for the calibration curves and for calculation of the fatty acid composition of oil samples were measured using a GC Solution system (Shimadzu Co. Ltd.).

Total amino acid analysis

Samples (0.5 g) were hydrolyzed with 15 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 110°C for 24 h. The vials were cooled and placed in a rotary evaporator at 45°C to remove HCl from the sample. The residue was then adjusted to pH 2.2 with a 0.2 M sodium citrate loading buffer (pH 2.2), diluted to a final volume of 25 mL with water, and filtered through a 0.45-μm Millipore membrane. The filtrate was analyzed using an amino acid analyzer (Shimadzu Co. Ltd.) equipped with a Shim-pack AMINO-Na column (6.0 × 100 mm). The buffer flow rate and OPA reagent flow rate were set to 0.6 mL/min and 0.3 mL/min, respectively. Fluorescent detection was achieved at 350 nm for excitation and 450 nm for emission.

Free amino acid analysis

Samples (5 g) were homogenized in a tissue grinder with 30 mL ethanol and incubated at 4°C for 24 h; the supernatant was collected after centrifugation at 1000 ×*g* for 15 min. The pellet obtained from the first centrifugation was mixed with 30 mL of 70% ethanol and re-centrifuged under the same conditions to collect the second supernatant. The final sample was obtained using a rotary evaporator to remove ethanol from the supernatant. The sample was mixed with 8 mL of distilled water and 0.2 g sulfosalicyclic acid. After 1 h of incubation at room temperature, the samples were centrifuged at 1000 ×*g* for 30 min and the pH of the supernatant was adjusted to 2.2 with 0.2 M lithium citrate loading buffer (pH 2.2). The samples were diluted to a final volume of 25 mL with water and filtered through 0.45 μm Millipore membranes. The filtrate was analyzed with an amino acid analyzer (Shimadzu Co. Ltd.) equipped with a Shim-pack AMINO-Li column (6.0 × 100 mm). The buffer flow rate and OPA reagent flow rate were set to 0.6 mL/min and 0.3 mL/min, respectively. Fluorescent detection was achieved at 350 nm for excitation and 450 nm for emission.

Statistical analysis

All mean values were analyzed via one-way analysis of variance (ANOVA). When differences were found among data, Duncan's multiple range test was used to compare the mean difference by using the statistical package for social sciences (SPSS) software package version 17 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at $p<0.05$.

Results and Discussion

Chemical composition

The proximate compositions with different ripening periods are shown in Table 1. Moisture content gradually decreased with ripening and significantly decreased at the 20th ripening day ($p<0.05$). Crude protein content significantly increased with ripening ($p<0.05$). These results were obtained due to bacteria or mold that produce small proteins and peptides. Little change could be observed in the crude lipid content; however, the ash content significantly increased with ripening ($p<0.05$). Carbohydrate content significantly decreased at ripening day 5, but carbohydrates were not detected after ripening day 10. The present study provides evidence that proximate analysis results vary during ripening.

Organic acid composition

Organic acid analyses with different ripening periods are shown in Table 2. The total organic acid level significantly increased on ripening days 10 (515.9 mg/100 g) and 15 (569.1 mg/100 g), but significantly decreased on ripening day 20 (435.1 mg/100 g) compared to that for

	Ripening day				
	0	5	10	15	20
Moisture	81.51 ± 2.40$^{a1)}$	80.45 ± 1.92^{ab}	79.33 ± 1.53^{ab}	78.69 ± 2.25^{ab}	77.25 ± 1.76^{b}
Crude protein	15.36 ± 0.34^{d}	16.44 ± 0.33^{c}	17.51 ± 0.37^{b}	17.94 ± 0.57^{b}	19.22 ± 0.38^{a}
Crude lipid	0.53 ± 0.01^{ab}	0.52 ± 0.01^{b}	0.54 ± 0.01^{ab}	0.55 ± 0.01^{a}	0.55 ± 0.01^{a}
Ash	2.44 ± 0.06^{d}	2.47 ± 0.08^{d}	2.62 ± 0.06^{c}	2.82 ± 0.06^{b}	2.98 ± 0.08^{a}
Carbohydrate$^{2)}$	0.16 ± 0.00^{a}	0.12 ± 0.00^{b}	-$^{3)}$	-	-

$^{1)}$ Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan's multiple range test.
$^{2)}$ Carbohydrate = 100 - (moisture + crude protein + crude lipid + ash).
$^{3)}$ Not detected.

Table 1: Proximate composition of skate during different ripening periods (%).

	Ripening day				
	0	5	10	15	20
Oxalic acid	142.99 ± 3.26^{d}1)	209.43 ± 5.59^{c}	250.10 ± 5.17^{b}	285.90 ± 6.75^{a}	2.21^{c}
Phosphoric acid	273.96 ± 5.39^{a}	161.63 ± 3.69^{c}	156.30 ± 4.37^{c}	181.89 ± 4.79^{b}	1.90^{b}
Citric acid	1.81 ± 0.04^{a}	-$^{2)}$	-	-	
Tartaric acid	0.72 ± 0.02^{e}	3.65 ± 0.08^{d}	9.85 ± 0.31^{c}	40.91 ± 1.22^{a}	0.62^{b}
Malic acid	2.77 ± 0.06^{a}	1.24 ± 0.03^{b}	0.84 ± 0.02^{c}	0.17 ± 0.00^{d}	0.00^{e}
Succinic acid	5.40 ± 0.13^{c}	8.50 ± 0.26^{a}	5.21 ± 0.12^{c}	6.36 ± 0.14^{b}	0.11^{d}
Lactic acid	59.53 ± 1.37^{c}	82.79 ± 2.30^{a}	74.09 ± 1.58^{b}	25.74 ± 0.77^{d}	0.07^{e}
Formic acid	0.26 ± 0.01^{e}	1.72 ± 0.04^{d}	3.28 ± 0.08^{c}	5.94 ± 0.12^{a}	0.10^{b}
Acetic acid	0.76 ± 0.02^{e}	7.77 ± 0.09^{d}	16.19 ± 0.32^{b}	22.19 ± 0.60^{a}	0.29^{c}
Total	488.20 ± 12.23^{c}	476.73 ± 9.29^{c}	515.86 ± 13.55^{b}	569.10 ± 11.73^{a}	6.14^{d}

$^{1)}$Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan's multiple range test.
$^{2)}$ Not detected.

Table 2: Organic acid contents of skate during different ripening periods (mg/100 g).

	Ripening				
	0	5	10	15	20
Sucrose	4.21 ± 0.12a1)	0.19 ± 0.00b	)		
Maltose	0.67 ± 0.01a	0.09 ± 0.00b	-		
Lactose	0.76 ± 0.02a	0.54 ± 0.01b	0.18 ± 0.00c		
Ribose	11.25 ± 0.28a	8.56 ± 0.27b	4.02 ± 0.10c	0.16 ± 0.00d	0.11 ± 0.00d
Mannose	0.37 ± 0.01a	0.09 ± 0.00b	0.07 ± 0.00c		
Galactose	0.21 ± 0.00a	0.05 ± 0.00c	0.06 ± 0.00b	0.06 ± 0.00b	
Xylose	0.17 ± 0.00a	-	-		
Glucose	6.97 ± 0.15a	0.83 ± 0.02b	0.6 ± 0.02c	0.09 ± 0.00d	
Total	24.61 ± 0.52a	10.35 ± 0.25b	4.93 ± 0.16c	0.31 ± 0.01d	0.11 ± 0.00d

$^{1)}$ Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan's multiple range test.
$^{2)}$ Not detected.

Table 3: Free sugar contents of skate during different ripening periods (mg/100 g).

the control (p<0.05). The oxalic acid content gradually increased until ripening day 15, but significantly decreased at ripening day 20 (p<0.05). The organic acid content of fish is known to be affected by the fishing method, amount of time since death, conditions after death, and prompt killing methods [23-25]. The lactic acid content of fish is low when they are promptly killed but is high after stressful or hard exercise [24]. Lactic acid was predominant, comprising approximately 90% of the total organic acid content in mackerel, pacific saury, yellow croaker, brown sole, wild and cultured red sea bream, rockfish, and flounder [23]. However, in the current study, the phosphoric acid content was the highest, followed by that of oxalic acid and lactic acid. Therefore, the pathways for rigor mortis may differ for bony and cartilaginous fish.

Free sugar composition

Free sugar analyses with different ripening periods are shown in Table 3. Among the 8 free sugars detected, the content of sucrose and maltose significantly decreased on ripening day 5 (p<0.05) and these sugars were not detected after ripening day 10. Lactose and mannose content significantly decreased until ripening day 10 (p<0.05), and these sugars were not detected after ripening day 15. Ribose content was significantly decreased with ripening until ripening day 15 (p<0.05), but no significant difference was observed between the content on ripening days 15 and 20. The galactose content significantly decreased by ripening day 5 (p<0.05) but the galactose content on ripening day 10 and 15 was lower than that for the control, significantly higher than that on ripening day 5 (p<0.05), and was not detected after ripening day 20. Xylose was detected in the control but not in the ripening samples. The glucose content was the highest in the control but significantly decreased until ripening day 15 and was not detected at ripening day 20. Glucose and ribose are the dominant free sugars in fish. Glucose is present in high concentrations in the muscle of live fish; glycogen decomposes after death, thereby increasing the glucose content [26]. Moreover, glucose levels in fish have been reported to change with the amount of time since death and conditions after death [27]. All the free

	Ripening					
		0	5	10	15	20
Saturated	12:00	0.02 ± 0.00^{b}1)	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
	14:00	1.16 ± 0.03^{a}	0.78 ± 0.02^{b}	0.48 ± 0.01^{c}	0.47 ± 0.01^{c}	0.46 ± 0.01^{c}
	16:00	12.00 ± 0.27^{e}	12.66 ± 0.29^{d}	13.99 ± 0.35^{c}	15.77 ± 0.39^{b}	16.54 ± 0.38^{a}
	17:00	0.69 ± 0.01^{b}	0.63 ± 0.02^{c}	0.75 ± 0.02^{a}	0.74 ± 0.02^{a}	0.72 ± 0.02^{a}
	18:00	4.78 ± 0.15^{b}	4.74 ± 0.11^{b}	6.88 ± 0.16^{a}	6.75 ± 0.15^{a}	6.65 ± 0.17^{a}
	20:00	0.08 ± 0.00^{e}	0.11 ± 0.00^{d}	0.17 ± 0.01^{c}	0.16 ± 0.00^{b}	0.18 ± 0.00^{a}
	21:00	0.61 ± 0.01^{a}	0.63 ± 0.01^{a}	0.45 ± 0.01^{b}	0.44 ± 0.01^{b}	0.43 ± 0.01^{b}
	22:00	0.41 ± 0.01^{b}	0.49 ± 0.01^{a}	0.33 ± 0.01^{c}	0.33 ± 0.01^{c}	0.32 ± 0.00^{c}
	24:00:00	9.57 ± 0.19^{b}	9.24 ± 0.21^{b}	11.04 ± 0.31^{a}	10.83 ± 0.29^{a}	11.18 ± 0.12^{a}
	Total	29.31 ± 0.67^{c}	29.31 ± 0.83^{c}	34.12 ± 0.83^{b}	35.50 ± 0.46^{a}	36.52 ± 0.78^{a}
Mono-unsaturated	14:1 n-5	0.02 ± 0.00^{b}	0.01 ± 0.00^{b}	0.37 ± 0.01^{a}	0.36 ± 0.01^{a}	0.36 ± 0.01^{a}
	16:1 n-7	3.73 ± 0.08^{a}	3.75 ± 0.09^{a}	2.59 ± 0.06^{b}	2.54 ± 0.06^{b}	2.51 ± 0.07^{b}
	18:1 n-9	7.65 ± 0.18^{b}	8.94 ± 0.27^{a}	6.45 ± 0.15^{c}	6.33 ± 0.14^{c}	6.24 ± 0.18^{c}
	20:1 n-9	0.07 ± 0.00^{b}	0.02 ± 0.00^{c}	0.35 ± 0.01^{a}	0.34 ± 0.01^{a}	0.34 ± 0.01^{a}
	22:1 n-9	0.21 ± 0.00^{b}	0.12 ± 0.00^{c}	0.27 ± 0.01^{a}	0.26 ± 0.01^{a}	0.26 ± 0.01^{a}
	24:1 n-9	0.03 ± 0.00^{c}	0.68 ± 0.01^{a}	0.37 ± 0.01^{b}	0.37 ± 0.01^{b}	0.36 ± 0.01^{b}
	Total	11.71 ± 0.29^{b}	13.53 ± 0.26^{a}	10.41 ± 0.27^{c}	10.21 ± 0.21^{cd}	10.06 ± 0.14^{d}
Di-unsaturated	18:2 n-6	1.17 ± 0.03^{a}	1.02 ± 0.03^{b}	0.78 ± 0.02^{c}	0.66 ± 0.01^{d}	0.66 ± 0.02^{d}
	20:2 n-6	0.31 ± 0.01^{a}	0.15 ± 0.00^{b}	0.10 ± 0.00^{c}	0.08 ± 0.00^{d}	0.03 ± 0.00^{e}
	22:2 n-6	0.15 ± 0.00^{a}	0.08 ± 0.00^{b}	0.04 ± 0.00^{c}	0.04 ± 0.00^{c}	0.04 ± 0.00^{c}
	Total	1.62 ± 0.02^{a}	1.25 ± 0.04^{b}	0.92 ± 0.03^{c}	0.78 ± 0.02^{d}	0.72 ± 0.01^{e}
Poly-unsaturated	18:3 n-3	10.72 ± 0.23^{a}	10.21 ± 0.22^{b}	8.27 ± 0.20^{c}	8.11 ± 0.21^{c}	7.99 ± 0.19^{c}
	20:3 n-6	0.01 ± 0.00^{c}	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{ab}
	18:3 n-6	0.12 ± 0.00^{a}	0.09 ± 0.00^{b}	0.08 ± 0.00^{c}	0.07 ± 0.00^{c}	0.07 ± 0.00^{c}
	20:4 n-6	6.80 ± 0.20^{a}	6.07 ± 0.11^{c}	6.58 ± 0.19^{ab}	6.46 ± 0.20^{b}	6.36 ± 0.14^{bc}
	20:5 n-3	0.02 ± 0.00^{d}	0.04 ± 0.00^{c}	0.06 ± 0.00^{a}	0.06 ± 0.00^{ab}	0.06 ± 0.00^{b}
	22:6 n-3	39.69 ± 0.92^{a}	39.48 ± 1.24^{a}	39.53 ± 0.91^{a}	38.77 ± 0.93^{a}	38.20 ± 0.76^{a}
	Total	57.36 ± 1.44^{a}	55.92 ± 1.09^{ab}	54.55 ± 1.43^{bc}	53.50 ± 1.10^{cd}	52.71 ± 0.74^{d}

Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan's multiple range test.

Table 4: Fatty acid composition of skate during different ripening periods (weight, %).

sugar levels were highest in the control and significantly decreased with ripening. In terms of the free sugar content in the skate, the level of ribose was the highest, followed by glucose and sucrose. Ribose was present until ripening day 20, galactose and glucose were present until ripening day 15, while lactose and mannose were present until ripening day 10. Ribose levels are considerably high in live fish muscle; ribose is isolated from inosine, the molecule obtained on ATP decomposition after death [26].

Fatty acid composition

Fatty acid analyses with different ripening periods are shown in Table 4. From the saturated fatty acids, the level of palmitic acid was the highest, followed by those of lignoceric acid and stearic acid. No significant differences were observed at ripening day 5 for lauric acid (C12:0), stearic acid (C18:0), and lignoceric acid (C24:0), but their levels significantly increased from ripening day 10 to 20 ($p<0.05$). The myristic acid (C14:0) level significantly decreased until ripening day 10 ($p<0.05$), but no significant differences were observed after this period. The palmitic acid (C16:0) and arachidic acid (C20:0) levels significantly increased with ripening ($p<0.05$). The heptadecanoic acid (C17:0) level significantly decreased at ripening day 5 but significantly increased after ripening day 10 ($p<0.05$). No significant difference was observed in heneicosanoic acid (C21:0) on ripening day 5, but a significant decrease was observed in the level from ripening day 10 to 20 ($p<0.05$). The level of behenic acid (C22:0) significantly increased at ripening day 5 ($p<0.05$), but significantly decreased after ripening day 10. Taken together, the saturated fatty acid levels significantly increased with increase in the duration of ripening ($p<0.05$). From the monounsaturated fatty acids, oleic acid (C18:1) and palmitoleic acid (C16:1) were the dominant forms. No significant differences were observed at ripening day 5 for myristoleic acid (C14:1) and palmitoleic acid but the myristoleic acid level significantly increased and the palmitoleic acid level significantly decreased from ripening day 10 to 20 ($p<0.05$). Oleic acid and nervonic acid (C24:1) levels significantly increased at ripening day 5 ($p<0.05$), while the oleic acid level significantly decreased and the nervonic acid significantly increased from ripening day 10 to 20 ($p<0.05$). Eicosenoic acid (C20:1) and erucic acid (C22:1) levels significantly decreased at ripening day 5, but significantly increased from ripening day 10 to 20 ($p<0.05$). Linoleic acid (C18:2) was the dominant form of di-unsaturated fatty acids. All di-unsaturated fatty acids significantly decreased with ripening ($p<0.05$). In the case of poly-unsaturated fatty acids, docosahexaenoic acid (C22:6) levels were the highest followed by □-linolenic acid (C18:3) and arachidonic acid (C20:4) levels. Poly-unsaturated fatty acid levels also significantly decreased with ripening ($p<0.05$).

Amino acid composition

The amino acid composition for different ripening periods is summarized in Table 5. Unlike land animals, fish have high protein requirements owing to low utilization of carbohydrates as an energy source. Therefore, the protein content in the body is an important energy source for fish and is also nutritionally important for normal growth [28]. The skate was found to contain high amounts of aspartic acid, glutamic acid, leucine, and lysine. Most amino acid levels increased with ripening except for some fluctuations in glycine, cysteine, and valine levels. The level of glutamic acid was the highest, followed by

	Ripening				
	0	5	10	15	20
Aspartic acid	1527.35 ± 44.99$^{b1)}$	1534.48 ± 36.71^{b}	1752.82 ± 33.76^{a}	1781.01 ± 50.90^{a}	1802.22 ± 41.13^{a}
Threonine$^{2)}$	740.21 ± 16.44^{d}	783.60 ± 15.63^{c}	852.09 ± 18.14^{b}	834.53 ± 26.29^{b}	888.54 ± 17.49^{a}
Serine	724.18 ± 16.39^{c}	769.84 ± 16.51^{b}	791.23 ± 10.04^{b}	793.12 ± 18.09^{b}	831.63 ± 19.03^{a}
Glutamic acid	2329.09 ± 58.42^{b}	2330.84 ± 73.62^{b}	2558.86 ± 61.10^{a}	2564.70 ± 58.43^{a}	2456.46 ± 65.36^{a}
Proline	620.39 ± 17.23^{c}	714.29 ± 14.37^{b}	741.68 ± 21.18^{b}	814.61 ± 18.38^{a}	793.08 ± 17.29^{a}
Glycine	624.45 ± 13.01^{c}	551.28 ± 13.63^{d}	693.65 ± 18.41^{b}	686.05 ± 20.55^{b}	781.34 ± 18.51^{a}
Alanine	881.80 ± 23.40^{c}	944.16 ± 26.17^{b}	964.70 ± 21.32^{b}	971.95 ± 19.83^{b}	1121.70 ± 25.75^{a}
Cystine	442.49 ± 9.81^{d}	572.17 ± 12.98^{b}	633.86 ± 15.86^{a}	643.50 ± 16.09^{a}	479.49 ± 11.11^{c}
Valine$^{2)}$	614.94 ± 12.91^{e}	659.55 ± 15.73^{d}	756.12 ± 23.82^{b}	719.27 ± 16.38^{c}	833.40 ± 20.06^{a}
Methionine$^{2)}$	468.07 ± 14.88^{c}	573.19 ± 13.27^{b}	574.75 ± 13.18^{b}	588.87 ± 12.74^{b}	658.12 ± 16.49^{a}
Isoleucine$^{2)}$	536.29 ± 11.26^{d}	632.76 ± 15.12^{c}	666.18 ± 20.12^{b}	654.92 ± 15.56^{bc}	745.56 ± 17.53^{a}
Leucine$^{2)}$	1143.01 ± 28.11^{c}	1385.55 ± 27.20^{b}	1364.98 ± 35.10^{b}	1385.89 ± 32.10^{b}	1451.89 ± 27.64^{a}
Tyrosine	428.94 ± 9.79^{c}	546.49 ± 14.60^{b}	545.34 ± 11.27^{b}	543.82 ± 12.83^{b}	587.60 ± 6.48^{a}
Phenylalanine$^{2)}$	485.92 ± 9.57^{c}	571.08 ± 13.03^{b}	586.59 ± 16.41^{b}	580.41 ± 15.28^{b}	641.28 ± 6.61^{a}
Histidine$^{2)}$	495.43 ± 11.33^{d}	542.02 ± 15.42^{c}	563.04 ± 13.75^{bc}	565.72 ± 7.39^{b}	589.03 ± 12.56^{a}
Lysine$^{2)}$	1642.29 ± 43.70^{c}	1914.07 ± 42.24^{b}	1929.84 ± 61.10^{b}	1941.10 ± 58.08^{b}	2046.92 ± 46.96^{a}
Arginine	836.61 ± 18.24^{c}	969.63 ± 22.74^{b}	982.25 ± 21.06^{b}	992.97 ± 22.26^{ab}	1028.61 ± 28.58^{a}
Total	14541.46 ± 344.49^{c}	15995.01 ± 486.39^{b}	16957.98 ± 383.33^{a}	17062.46 ± 372.28^{a}	17736.87 ± 516.11^{a}

1) Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan's multiple range test.
2) Essential amino acid

Table 5: Total amino acid contents of skate during different ripening periods (mg/100 g).

	Ripening				
	0	5	10	15	20
Phosphoserine	0.76 ± 0.02^{d}1)	1.26 ± 0.03^{a}	0.97 ± 0.02^{b}	0.41 ± 0.01^{e}	0.81 ± 0.02^{c}
Taurine	65.65 ± 1.46^{a}	61.00 ± 1.22^{b}	54.19 ± 1.15^{c}	41.35 ± 1.30^{d}	31.28 ± 0.62^{e}
Phosphoethanolamine	0.63 ± 0.01^{d}	3.70 ± 0.08^{c}	4.79 ± 0.06^{ab}	4.92 ± 0.11^{a}	4.67 ± 0.11^{b}
Urea	1041.08 ± 26.11^{a}	237.46 ± 7.50^{b}	31.64 ± 0.76^{c}	22.34 ± 0.51^{c}	19.63 ± 0.52^{c}
Aspartic acid	12.85 ± 0.36^{a}	9.12 ± 0.18^{b}	7.19 ± 0.21^{c}	5.09 ± 0.11^{d}	1.34 ± 0.03^{e}
Threonine	24.12 ± 0.50^{a}	11.31 ± 0.28^{b}	3.61 ± 0.10^{c}	0.46 ± 0.01^{d}	0.39 ± 0.01^{d}
serine	31.99 ± 0.85^{a}	22.19 ± 0.62^{b}	11.82 ± 0.26^{c}	2.78 ± 0.06^{d}	0.56 ± 0.01^{e}
Asparagine	0.60 ± 0.01	2)	-	-	-
Glutamic acid	11.35 ± 0.24^{a}	9.52 ± 0.23^{b}	3.48 ± 0.11^{c}	1.48 ± 0.03^{d}	1.69 ± 0.04^{d}
Sarcocine	230.44 ± 7.33^{a}	195.60 ± 4.53^{b}	164.14 ± 3.76^{c}	168.69 ± 3.65^{c}	154.09 ± 3.86^{d}
?-aminoadipic acid	3.28 ± 0.07^{b}	3.80 ± 0.09^{a}	3.17 ± 0.10^{b}	2.64 ± 0.06^{c}	1.79 ± 0.04^{d}
Proline	17.23 ± 0.42^{a}	9.44 ± 0.19^{c}	10.75 ± 0.28^{b}	9.23 ± 0.21^{c}	7.88 ± 0.15^{d}
Glycine	27.89 ± 0.64^{a}	24.32 ± 0.65^{b}	24.16 ± 0.50^{b}	22.78 ± 0.54^{c}	19.59 ± 0.22^{d}
Alanine	23.73 ± 0.47^{b}	24.79 ± 0.57^{b}	31.68 ± 0.89^{a}	31.51 ± 0.83^{a}	20.22 ± 0.21^{c}
Citrulline	3.20 ± 0.07^{c}	3.64 ± 0.10^{a}	3.09 ± 0.08^{c}	3.44 ± 0.04^{b}	2.82 ± 0.06^{d}
?-aminobutyric acid	0.30 ± 0.01^{d}	0.31 ± 0.01^{d}	0.36 ± 0.01^{c}	0.42 ± 0.01^{b}	0.44 ± 0.01^{a}
Valine	5.65 ± 0.12^{a}	4.78 ± 0.11^{b}	5.56 ± 0.12^{a}	5.59 ± 0.13^{a}	3.50 ± 0.10^{c}
Cystine	0.00 ± 0.00^{e}	0.43 ± 0.01^{c}	0.34 ± 0.01^{d}	0.67 ± 0.01^{b}	0.90 ± 0.03^{a}
Methionine	4.29 ± 0.10^{a}	2.53 ± 0.07^{b}	2.32 ± 0.05^{c}	1.02 ± 0.03^{d}	0.25 ± 0.01^{e}
Isoleucine	3.47 ± 0.08^{a}	2.27 ± 0.05^{b}	2.33 ± 0.05^{b}	1.56 ± 0.03^{c}	0.59 ± 0.02^{d}
Leucine	6.41 ± 0.15^{a}	4.38 ± 0.05^{b}	4.42 ± 0.09^{b}	3.67 ± 0.10^{c}	1.19 ± 0.03^{d}
Tyrosine	3.02 ± 0.08^{a}	2.33 ± 0.05^{c}	2.87 ± 0.08^{b}	1.69 ± 0.03^{d}	-
phenylalanine	3.44 ± 0.08^{a}	3.40 ± 0.09^{ab}	3.29 ± 0.07^{b}	2.97 ± 0.05^{c}	-
?-alanine	32.62 ± 0.62^{a}	26.71 ± 0.56^{b}	21.46 ± 0.36^{cd}	22.20 ± 0.47^{c}	20.96 ± 0.38^{d}
?-amino-n-butyric acid	0.22 ± 0.00^{c}	0.45 ± 0.01^{b}	0.44 ± 0.01^{b}	0.52 ± 0.01^{a}	0.10 ± 0.00^{d}
Histidine	4.53 ± 0.05^{a}	3.36 ± 0.11^{b}	3.42 ± 0.10^{b}	3.15 ± 0.07^{c}	1.28 ± 0.02^{d}
1-methylhistidine	0.21 ± 0.00	-	-	-	-
Carnosine	0.68 ± 0.02^{b}	0.72 ± 0.02^{a}	0.36 ± 0.01^{c}	0.26 ± 0.01^{d}	0.25 ± 0.01^{d}
Anserine	0.14 ± 0.00^{b}	0.11 ± 0.00^{c}	0.18 ± 0.00^{a}	0.11 ± 0.00^{c}	0.05 ± 0.00^{d}
Ornitine	3.99 ± 0.12^{c}	4.53 ± 0.09^{b}	4.93 ± 0.14^{a}	4.10 ± 0.12^{c}	2.50 ± 0.05^{d}
Lysine	11.96 ± 0.24^{a}	9.95 ± 0.23^{b}	9.27 ± 0.21^{c}	7.31 ± 0.15^{d}	2.99 ± 0.03^{e}
Ethanolamine	3.16 ± 0.07^{b}	2.52 ± 0.08^{c}	1.65 ± 0.04^{d}	0.76 ± 0.02^{e}	4.11 ± 0.08^{a}
Arginine	3.23 ± 0.09^{a}	2.56 ± 0.06^{b}	2.14 ± 0.07^{d}	2.32 ± 0.07^{c}	1.52 ± 0.03^{e}
Ammomia	53.28 ± 2.54^{e}	1133.72 ±22.12^{d}	1269.45 ± 27.32^{c}	1307.02 ± 20.22^{b}	1449.96 ± 33.59^{a}
Total	1582.11 ± 34.50^{a}	688.48 ± 16.15^{b}	420.04 ± 9.00^{c}	375.41 ± 8.42^{d}	307.50 ± 8.54^{e}

1) Values are means ± SD (n = 3). Values with different superscripts in the same row are significantly different at P<0.05 by Duncan's multiple range test.
2) Not detected.

Table 6: Free amino acid contents of skate during different ripening periods (mg/100 g).

those of lysine, aspartic acid, and leucine. No significant differences were observed on ripening day 5 for aspartic acid and glutamic acid, but the levels significantly increased from ripening day 10 to 20 ($p<0.05$). The threonine, serine, proline, alanine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine levels, as well as the total amino acid content, significantly increased with ripening ($p<0.05$). The glycine, valine, and cysteine levels also significantly increased with ripening ($p<0.05$) except on ripening days 5, 15, and 20, respectively. The levels of all the essential amino acids also increased with increase in the ripening period. Taken together, the total amino acid content increased to a level approximately 22% greater than that in the control over 20 ripening days.

Free amino acid composition

The contents of individual free amino acids with different ripening periods in skate are shown in Table 6. Free amino acids are important components of bioactive material and impart a characteristic taste to food. In fish, free amino acids are used as a chemical signal to influence behavior, communication, and physiological metabolism [29]. The major free amino acids found throughout the ripening period were urea, sarcosine, taurine, and β-alanine. Although the contents of some individual free amino acids in the skates fluctuated during the ripening period, the levels of most free amino acids, as well as total free amino acids, significantly decreased with ripening. However, the levels of phosphoethanolamine, α-aminobutyric acid, and ammonia significantly increased with ripening ($p<0.05$). Asparagine and 1-methylhistidine were detected in the control, but not in the ripening groups. When compared to the control, the ripening groups showed more than 90% reduction in the urea, aspartic acid, threonine, serine, asparagine, methionine, tyrosine, phenylalanine, and 1-methylhistidine levels over the 20-day ripening period.

In conclusion, we analyzed the nutritional composition changes during different ripening periods. Total ripening increased protein and ash and total organic acid content with the later only were increasing until the 15th day. Moisture content generally reduced during ripening. Moreover, it was shown that ripening over a period of 20 days changed profiles of organic acids, free sugars, fatty acids, and total proteins. These results suggest fundamental data on the nutritional composition of the Korean traditional food, ripened skate.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0077476).

References

1. Itou K, Akahane Y (2000) Changes in proximate composition and extractive components of rice-bran-fermented mackerel Heshiko during processing. Fish Sci 66: 1051-1058.
2. Sazaki J (2003) Traditional taste refined by the proper environment – Funazushi. J Jap Diet Life 14: 61-65.
3. Yoon JH, Lee KC, Weiss N, Kang KH, Park YH (2003) Jeotgalicoccus halotolerans gen. nov., sp. nov. and Jeotgalicoccus psychrophilus sp. nov., isolated from the traditional Korean fermented seafood jeotgal. Int J Syst Evol Microbiol 53: 595-602.
4. Sato K, Yoshinaka R, Sato M, Shimizu Y (1986) Collagen content in the muscle of fishes in association with their swimming movement and meat texture. B Jpn Soc Sci Fish 52: 1595-1600.
5. Smith CP, Wright PA (1999) Molecular characterization of an elasmobranch urea transporter. Am J Physiol 276: R622-626.
6. Suyama M, Suzuki H (1985) Nitrogeous constituents in the muscle extracts of marine elasmobranchs. B Jpn Soc Sci Fish 41: 787-790.
7. Ando M, Toyohara H, Sakaguchi M (1992) Post-mortem tenderization of rainbow trout muscle caused by the disintegration of collagen fibers in the pericellular connective tissue. B Jpn Soc Sci Fish. 58: 567-570.
8. Ando M, Toyohara H, Shimizu Y, Sakaguchi M (1993) Postmortem tenderization of fish muscle due to weakening of pericellular connective tissue. B Jpn Soc Sci Fish 59: 1073-1076.
9. Hatae K, Tamari S, Miyanaga K, Matsumoto J (1985) Species differences and changes in the physical properties of fish muscle as freshness decreases. B Jpn Soc Sci Fish 51: 1155-1161.
10. Cremer MA, Terato K, Seyer JM, Watson WC, O'Hagan GO, et al. (1991) Immunity to type XI collagen in mice. Evidence that the alpha 3(XI) chain of type XI collagen and the alpha 1(II) chain of type II collagen share arthritogenic determinants and induce arthritis in DBA/1 mice. J Immunol 146: 4130-4137.
11. Jeong CH (1999) A review of taxonomic studies and common names of Rajid fishes (Elasmobranchii, Rajidae) from Korea. Kor J Ichthyol 11: 198-210.
12. Kim HJ, Eo JH, Kim SJ, Eun JB (2010) Physicochemical changes in fermented skate (Raja kenojei) treated with organic acids during storage. Kor J Food Sci Technol 42: 438-444.
13. Kim KH, Cho HS (2008) The physicochemical and sensory characteristics of Jook containing different levels of skate (Raja kenojei) flour. J East Asian Soc Diet Life 18: 207-213.
14. Nam HK, Lee MK (1995) Studies on the fatty acids and cholesterol level of Raja Skate. J Kor Oil Chem Soc 12: 55-58.
15. Hwang JH, Mizuta S, Yokoyama Y, Yoshinaka R (2007) Purification and characterization of molecular species of collagen in the skin of skate (Raja kenojei). Food Chem 100: 921-925.
16. Jin CH, Yang U, Kim SH, Ryu JW, Lee JC, et al. (2007) The Protective effect of chondroitin from Raja kenojei cartilage on collagen-induced arthritis in DBA/1J mice. Food Sci Biotechnol 16: 594-599.
17. Mizuta S, Hwang JH, Yoshinaka R(2002) Molecular species of collagen from wing muscle of skate (Raja kenojei). Food Chem 76: 53-58.
18. Mizuta S, Hwang JH, Yoshinaka R (2003) Molecular species of collagen in pectoral fin cartilage of skate (Raja kenojei). Food Chem 80: 1–7.
19. Shon JH, Eo JH, Hwang SJ, Eun JB (2011) Effect of processing conditions on functional properties of collagen powder from skate (Raja kenojei) skins. Food Sci. Biotechnol 20: 99-106.
20. AOAC (1999) Official methods of Analytical chemistry (16th edn). Arlington: The Association of Official Analytical Chemists, Inc.
21. Mikami H, Ishida Y (1983) Post-column fluorometric detection of reducing sugars in high performance liquid chromatography using arginine. Bunseki Kagaku 32: 207-210.
22. Iverson SJ, Lang SL, Cooper MH (2001) Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 36: 1283-1287.
23. Hwang JH, Lee SW, Rha SJ, Han KH, Kim SJ (2012) Nutritional characteristics of juvenile black rockfish Sebastes schlegeli fed a diet of fig leaf extract. Kor J Fish Aquat Sci 45: 570-578.
24. Kim HY, Shin JW, Park HO, Choi SH, Jang YM, et al. (2000) Comparison of taste compounds of red sea bream, rockfish and founders differing in the localities and growing conditions. Kor J Food Sci Technol 32: 550-563.
25. Shim KH, Lee JH, Ha YL, Choi SD, Seo KI, et al. (1994) Changes of organic acid contents on heating conditions of fishes. J. Kor. Soc. Food Nutri. 23: 939-944
26. Park Y, Jang D, Kim S (1997) Processing of the Sea Food, Hyungsul Press, Seoul, Korea, 166-168.
27. Kim HY, Kim EH, Kim DH, Oh MJ, Shin TS (2009) The nutritional componnents of olive flounder (Paralichthys olivaceus) fed diet with yuza (Citrus junos Sieb ex Tanaka). Kor J Fish Aquat Sci 42: 215-223.
28. Wilson RP (2002) Amino acids and Proteins: In "Fish Nutrition" (3th edn). John EH and Ronald WH, eds. Academic press, San Diego, California, USA, 143-177.
29. Saglio PH, Fauconneau B, Blanc JM (1990) Orientation of carp, Cyprinm carpio L., to free amino acids from Tubifex extract in an olfactometer. J Fish Biol 37: 887-898.